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AMENDED CLAIMS

1. A method of transferring a nucleic acid of interest across a biological membrane, and/or direction thereof to a specific location within or on a cell, by use of a synthetic transport entity; which comprises the steps of
- (a) providing a carrier molecule comprising the nucleic acid of interest and a binding element (BE) target sequence, said BE target sequence being a peptide nucleic acid (PNA) target;
 - (b) providing a complex by coupling at least one functional element (FE) to a binding element (BE), said BE being a peptide nucleic acid (PNA) or a derivative or an analogue thereof;
 - (c) hybridising the BE of said complex to the BE target of said carrier; and
 - (d) contacting said transport entity with said biological membrane to provide for a transfer of the nucleic acid of interest across said membrane.
2. A method according to claim 1, wherein in step (b), a complex is provided, wherein said BE and FE(s) are separated by linker element(s).
3. A method according to claim 1 or 2, wherein in step (a), the carrier provided is a plasmid or an oligonucleotide vector comprising said nucleic acid of interest and at least one BE target sequence.
4. A method according to any one of the preceding claims, wherein in step (a), a detectable marker element is also inserted in said carrier.
5. A method according to any one of the preceding claims, wherein the nucleic acid of interest is a gene encoding a peptide, a protein or an RNA.
6. A method according to any one of the preceding claims, wherein the biological membrane is a cell wall.
7. A method according to claim 6, wherein in step (b), an FE comprising an antennapedia peptide is provided in said complex, which enables said transfer of the nucleic acid of interest across the cell wall.
8. A method according to any one any one of claims 1-5, wherein the biological membrane is a nuclear membrane.

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9. A method according to claim 8, wherein in step (b), an FE comprising a nuclear localization signal is provided in said complex, which enables said transfer of the nucleic acid of interest across a nuclear membrane.

sub 24 → 10. A method according to any one of claims 1-5, wherein in step (b), ~~an FE comprising a protein, such as an HIV protein, e.g. TAT, is provided in said complex, which enables both membrane translocation and nuclear transport of the nucleic acid of interest.~~

11. A kit comprising components for making a transport entity capable of for transferring a nucleic acid of interest across a biological membrane, and/or direction thereof to a specific location within or on a cell, which kit comprises a binding element (BE) in the form of a peptide nucleic acid (PNA) or a derivative or an analogue thereof, a functional element (FE); an oligonucleotide comprising a target for said BE suitable for cloning in a desired plasmid containing said nucleic acid of interest; and optionally reagents suitable for such transfer and/or direction.

12. A kit according to claim 11, wherein the functional element (FE) is an antennapedia peptide.

13. A kit according to claim 11, wherein the functional element (FE) is a nuclear localisation signal (NLS), such as a SV40 large T antigen protein, or a fragment thereof exhibiting nuclear localizing signal properties.

14. A kit according to claim 11, wherein the functional element (FE) is a protein enabling both membrane translocation and nuclear transport.

sub 25 → 15. A synthetic transport entity suitable for use in the method according to any one of claims 1-10, which is comprised of at least one functional element (FE), which is complexed to a binding element (BE) in the form of a peptide nucleic acid (PNA) or a derivative or an analogue thereof, and a nucleic acid carrier, which comprises at least one BE target sequence and a nucleic acid of interest in a vector; said complex ~~being hybridised to said carrier using the BE-BE target interaction.~~

16. A transport entity according to claim 15, wherein said vector is a plasmid or an oligonucleotide.

17. A transport entity according to claim 15 or 16, wherein the carrier includes a detectable marker element.

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18. A transport entity according to any one of claims 15-17, wherein the nucleic acid of interest is a gene encoding a peptide, a protein or an RNA.

19. A transport entity according to any one of claims 15-18, wherein said BE and FE(s) are separated by linker element(s).

20. A transport entity according to any one of claims 15-19, which comprises more than one FE-BE-complex, each one of which is hybridised to a separate BE target sequence present on the same carrier.

21. A transport entity according to claim 20, wherein each FE-BE-complex comprises two or more FEs, preferably spaced by linkers.

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22. A transport entity according to any one of claims 15-21, wherein the FE is an antennapedia peptide.

23. A transport entity according to any one of claims 15-21, wherein the FE is a nuclear localization signal (NLS), such as a SV40 large T antigen protein, or a fragment thereof exhibiting nuclear localizing signal properties.

24. A transport entity according to any one of claims 15-21, wherein the FE is a protein, such as an HIV protein, e.g. TAT, exhibiting properties enabling both membrane translocation and nuclear transport.

25. A recombinant cell comprising one or more genetic modification(s) provided by use of the method as defined in any one of claims 1-10 or a transport entity as defined in any one of claims 15-24.

26. Use of a transport entity according to any one of claims 15-24 or a cell according to claim 25 in gene therapy.

27. Use of a transport entity according to any one of claims 15-24 or a cell according to claim 25 in DNA-vaccination.

No Bk
Signed

Add (1)